light, or using a visible light initiated system comprising the dye ethyl eosin and cocatalyst triethanol amine and exposing the sample to green light in the region of 500-580nm. A small quantity of bifunctional crosslinkers may also be added, e.g., tetraethylene glycol diacrylate. The degree of substitution of available sites by polymerizable groups is varied depending on the degree of crosslinking and drug loading required. The presently preferred ratio of unsaturated groups to all available sites is between 0.04 and 0.75. A more preferred range is between 0.1 to 0.5.

The preferred mode of drug delivery for the soluble derivatives of taxol is intravenous. The polymer-drug conjugate is dissolved in normal saline or a physiological buffer and infused intravenously.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLES

Example 1

Taxol-PEG: PEG at both 2' and 7 position coupled with a relatively stable urethane linkage.

10 mg (0.0117 mmol) of Taxol (Sigma Chemical) was dissolved in 5 ml of chloroform and a ten fold excess of 1,1-carbonyldiimidazole (CDI, 18.95 mg., 0.117 mmol) was added to activate the hydroxyl (OH) groups on the Taxol molecule. (There are three OH groups on Taxol **(compound 1)** at the 1, 7, and the 2' positions. The OH at the 2' position is the most labile followed by the OH at the 7 position while the OH at the 1 position is sterically hindered and nonreactive. This procedure resulted in the formation of the Taxol-CDI **(2)** derivative at the 2' and 7

positions). The reaction was allowed to proceed for 2 hours at room temperature and then extracted in water 3 times to remove the excess CDI and imidazole formed as a reaction product and then dried over anhydrous sodium sulfate. Monomethoxy polyethylene glycol amine (MPEG-amine, **14**) of molecular weight 5000 g/mol was added (58.5 mg, 0.117 mmol) to the reaction mixture and allowed to react for 2 hours with stirring at room temperature. The product was extracted with 10% aqueous potassium nitrate, dried over anhydrous sodium sulfate and evaporated *in vacuo* or freeze dried.

UV spectroscopy (Shimadzu Instruments) was utilized to determine if Taxol had been derivatized and if a water-soluble PEG-Taxol derivative (**3**) substituted at the 2' and/or 7 position was obtained. Taxol was added to water (in an attempt to see any solubility that may result) to give a suspension which was filtered and a scan of this sample was obtained. No characteristic absorbances for taxol were seen implying a negligible solubility below the detection limit of the instrument. A scan of taxol dissolved in ethanol, however, showed the characteristic absorbance in the region of 240 nm and a shoulder around 270-280 nm. Next, MPEG-amine was dissolved in water and showed negligible absorption in the region of interest. A scan of the freeze dried product (PEG-taxol derivative) was added to water (100% of this product did not dissolve in water) and filtered. It showed the characteristic absorption of taxol indicating that the drug was solubilized in water as a result of the coupling reaction with PEG.

Example 2

Taxol-PEG: PEG at 7 position coupled with a relatively stable urethane linkage.

It is known that the C13 ester side chain and the 2'-hydroxyl group on the side chain are essential for biological activity (Mathew et al., 1992, *J. Med. Chem.* 35:145-151). The introduction of a substituent at the 2' position has resulted in a loss in the ability to promote microtubule assembly. Derivatives of taxol having a substituent at the 7 position however, retain their ability to alter cell proliferation and microtubule polymerization. The following procedure describes a method to obtain water-soluble PEG derivatives of taxol having the PEG substituent at the 7 position and not the 2' position.

The 2'-hydroxyl on taxol was first protected using the [(2,2,2-trichloroethyl)oxy]carbonyl, or 'troc' protective group. Taxol (50 mg) in chloroform (5 ml) and pyridine (0.1 ml) was cooled to -20°C and treated with 2,2,2-trichloroethyl chloroformate (0.008 ml) for 45 minutes. Workup by standard methods yielded the 2'-troc derivative (4) together with small amounts of taxol and 2', 7-bis troc taxol. The product could be isolated by TLC with ethyl acetate-hexane (1:1) as solvent: yield 85%.

The product 4 was reacted as in example 1 to obtain the 2'-troc-7-MPEG taxol (5). The protective troc group was then removed by dissolving 5 in 2 ml methanol-acetic acid (9:1) and addition of zinc dust (40 mg). The mixture was stirred for 10 minutes at room temperature, filtered to remove excess zinc and the 7-PEG taxol (6) obtained by precipitation with diethyl ether and drying *in vacuo*.

Example 3

Water-soluble Prodrugs of Taxol: PEG at the 2' Position Bound by a Hydrolyzable Linkage (Reaction of Taxol 2'-OH with PEG-COOH)

A PEG derivative of Taxol at the 2'-OH position will make taxol soluble in aqueous medium. An ester at this position will be hydrolyzed to give back taxol in its active form. This strategy is utilized in the delivery of taxol in a water-soluble form. This form is called the 'prodrug'.

Preparation of MPEG-1900-COOH: MPEG-1900 (9.5g, 5 mmol) was dissolved in toluene (50ml) and dried by distilling off most of the toluene; succinic anhydride (20g, 20mmol) was added and the mixture was stirred for 5 hours on an oil bath at 150°C. The mixture was cooled, taken up in dichloromethane and precipitated in ether. The product **(15)** was reprecipitated twice using dichloromethane/ether; yield 8.5g (85%). Homologues of succinic anhydride, e.g., glutaric anhydride, may be utilized for this reaction.

Preparation of MPEG-Taxol Ester (7): MPEG-COOH (0.5 mmol COOH) and taxol (0.55mmol) were added to dichloromethane (20ml), and dicyclohexyl carbodiimide (DCC, Aldrich, 0.65mmol), and 4-dimethyl aminopyridine (DMAP, Aldrich, 0.125mmol) were added as coupling agents. The mixture stirred for 3-4 hours at room temperature, the precipitate of dicyclohexyl urea (DCU) filtered and the filtrate evaporated to dryness in vacuum. The residue was extracted with acetone and the product 7 precipitated by ether; yeilds 70-85%.

Example 4

Water-Soluble Prodrugs of Taxol: PEG at the 2' Position Bound by a Hydrolyzable Linkage (Reaction of Taxol 2'-COOH with PEG-OH)

An alternate strategy to the one in example 3 is to make a carboxylic acid derivative of taxol and esterify

it with untreated PEG, i.e., PEG having available OH groups. The carboxylic acid derivatives of taxol are prior art and have been synthesized by esterification with succinic or glutaric anhydrides (Deutsch et al., 1989; *J. Med. Chem.* 32:788-792).

Preparation of 2'-Succinyl Taxol (8): Taxol (0.5g, 0.59 mmol) and succinic anhydride (0.90g, 7.6mmol) in 12 ml of pyridine were allowed to react for 3 hours at room temperature after which the mixture was evaporated to dryness *in vacuo*. The residue was treated with 20ml of water, stirred for 20min and then filtered. The precipitate was dissolved in acetone, water slowly added, and the fine crystals of **8** were collected; yield (75-85%).

Preparation of 2'-Glutaryl Taxol (9): A similar procedure used for **8** by reaction of taxol with glutaric anhydride gave 85-95% yield of the derivative **9** when recrystallized from chloroform/benzene.

Preparation of MPEG-Taxol Ester (10, 11): Essentially the identical procedure used for preparation of product 7 was used here, the MPEG-COOH being replaced by untreated MPEG (13) and taxol by products **8** or **9** to obtain respectively the products **10** and **11**. Yields obtained were in the range of 75-85%. The product **10** was identical to the product 7 obtained in example 3.

Homologues of **8** and **9** obtained by reaction of taxol with homologues of cyclic anhydrides such as succinic anhydride may be utilized for preparation of PEG-Taxol esters.

Example 5

Water-Soluble Taxol-PEG Derivatives: Use of Branched Chain or 'Star' PEGs for a Multiplicity of

Attachment Sites for Taxol.

The use of derivatives in examples 1 through 4 describes the covalent attachment of taxol to PEGs that are monofunctional (1 available attachment site per molecule) such as MPEG or at most, those that are bifunctional (regular PEGs, 2 available attachment sites per molecule). In order to increase the efficiency of drug delivery in terms of increasing the ratio of the mass of drug to the mass of inactive carrier (in this case PEG), it is desirable to utilize a molecule (carrier) that for a given molecular weight, can carry several molecules of drug as opposed to one or two molecules per molecule of carrier.

Preparation of Branched PEG-Taxol: Branched polymers of PEG produced by polymerization of a monoacrylate or monomethacrylate derivative were also used for covalent attachment of taxol. The monoacrylate **(16)** and monomethacrylate **(17)** derivatives of PEG (Macrochem Labs) of molecular weights ranging from 1000 to 20000 having one free hydroxyl end group were polymerized in solution to obtain branched polymers of a given molecular weight. 10g (5mmol) of PEG 2000 monoacrylate was dissolved in 100 ml of dry toluene. A thermal free radical initiator 2,2'-azobis isobutyronitrile (AIBN) was added 0.016g (0.1 mmol) and the solution heated to 80°C. The reaction was allowed to proceed overnight and the polymer precipitated from solution with diethyl ether. The polymer was further purified by redissolution in toluene and reprecipitation with ether and dried *in vacuo*. The branched PEG so obtained was then coupled to taxol by the techniques illustrated in examples 1-4. Those skilled in the art will recognize that other free radical initiators or initiating mechanisms, and other PEG derivatives, e.g., vinyl ethers of PEG, may be utilized to obtain branched molecules.

Preparation of Star PEG-Taxol: 'Star' PEG specifically 8-arm PEG, MW 22800 (Macrochem Labs) was used as obtained. This PEG had 8 potential coupling sites per molecule. Other such PEGs may also be used with a greater number of arms and higher molecular weight preferably less than 100000 to allow clearance from the body by the kidneys. The reactions described in examples 1 through 4 were utilized to covalently attach drugs such as taxol to these molecules.

Example 6

Water-Soluble Taxol-PEG Derivatives: Use of Copolymers of PEG and Functional Monomers for a Multiplicity of Attachment Sites for Taxol.

Another strategy for drug delivery is the copolymerization of functional monomers with polymerizable derivatives of PEG such as MPEG-acrylate **(18)** or methacrylate **(19)** or the corresponding 'mono' derivatives of regular PEG. Examples of functional monomers used in the synthesis are those bearing the carboxyl group, e.g., acrylic acid, vinyl acetic acid (3-butenoic acid) and higher homologues; those bearing the amine group, e.g., allyl amine and higher homologues; those bearing the hydroxyl group, e.g., allyl alcohol (2-propene-1-ol) and higher homologues; allyl chloride (3-chloropropene), other unsaturated halides and corresponding higher homologues, and unsaturated compounds bearing aldehyde groups. The presence of these pendant functional groups allows for the attachment of a wide range of drugs possessing different functionalities.

Copolymerization of MPEG-acrylate and Acrylic Acid: MPEG-acrylate was prepared by reaction of MPEG (Nippon Oil and Fat) with acryloyl chloride (Aldrich). MPEG-5000 (10g, 2mmol) was dissolved in toluene (150 ml)

and approximately 50 ml of toluene was distilled over to ensure removal of water. Acryloyl chloride (4mmol) that had been distilled immediately prior to use, was added after the MPEG/toluene had been cooled on an ice bath. Triethylamine (4mmol, not essential) was added and the reaction mixture refluxed for 4 hours. Triethylamine hydrochloride, formed as a by product was filtered and the MPEG-acrylate precipitated with by addition of excess ether to the filtrate. The product was purified by reprecipitation from toluene and then dried *in vacuo*: Yield 90%.

Acrylic acid was vacuum distilled prior to use. MPEG-acrylate (1mmol) and acrylic acid (1mmol) were dissolved in dry toluene and the free radical thermal initiator AIBN (0.05mmol) added. The reaction mixture was heated to 80°C, the reaction was allowed to proceed overnight and the polymer precipitated from solution with diethyl ether. The polymer was further purified by redissolution in toluene and reprecipitation with ether and dried *in vacuo*. The copolymer containing labile carboxylic acid groups was then coupled to taxol by the technique described for product 7.

Example 7

Hydrogels Containing Bound Taxol for Sustained Release Drug-delivery.

Star (8-arm) PEG-22800 (2.85g, containing 1 mmol OH groups) was dissolved in 25 ml of dry toluene. The solution was cooled on an ice bath and 0.33mmol of freshly distilled acryloyl chloride was added and the reaction mixture kept at 70°C for 4 hours. The toluene was removed by vacuum distillation and the partial acrylate derivative redissolved in 25ml of dry dichloromethane. 2'-succinyl taxol (0.8 mmol, prepared as in example 4), DCC (0.95 mmol)

and DMAP (0.18 mmol) were added to the reaction mixture which was stirred at room temperature for 3-4 hours. The precipitate of DCU was filtered and the PEG-partial acrylate derivatized with taxol (star-PEG-acrylate-taxol, SPAT) was precipitated in excess ether and dried *in vacuo*. Yield: 70-85%

This derivative could be crosslinked by free radical polymerization in organic solvent or in aqueous medium by addition of the appropriate initiators. In organic solvent, the UV photoinitiator DMPA was used. A 10 wt% solution of SPAT in dichloromethane containing 0.05% DMPA was poured in an open stainless steel mold in the shape of a small disc 1cm diameter and thickness 0.5cm and exposed to long-wave UV radiation from a mercury arc lamp. The solution gelled within 30 seconds. The gel was freeze dried. When placed in water of aqueous medium, the gelled discs would swell and imbibe water.

A similar crosslinking procedure was utilized in aqueous buffer except the photoinitiator in this case was water-soluble UV initiator AAPH at 0.05% concentration. Other organic soluble initiators such as benzil, and other aqueous photoinitiating systems such as the ethyl eosin, triethanol amine system were also successful.

A branched PEG polymer, or a branched copolymer could be utilized instead of the star polymer to achieve a similar drug immobilized gel.

CLAIMS

What is claimed is:

1. A Taxol derivative having the general formula R_1-T-R_2:

wherein R_1 = X-P or -OH, and R_1 is located on the 2'-carbon of the taxol side chain;

wherein R_2 = -X-P or -OH, and R_2 is located on the 7-carbon atom of the ring structure;

but R_1 and R_2 are not simultaneously OH;

and the drug taxol T, is covalently linked to a water-soluble polymer P, through a covalent linking group X.

2. The composition of claim 1 in which said covalent linking group X is selected from ester, diester, urethane, amide, secondary or tertiary amine, or ether groups.

3. The composition of claim 1 in which said water-soluble polymer P is based on polyethylene glycol.

4. The composition of claim 3 in which said polyethylene glycol is a linear monofunctional polyethylene glycol, wherein:

$R_1 = R_9(CH_2CH_2O)_nCH_2CH_2X$- or -OH, and R_9 is selected from alkoxy or aryloxy;

R_2 = -R_1 or -OH;

and n is a number between, and including, 5 and 2500.

5. The composition of claim 3 in which the polyethylene glycol is a linear bifunctional polyethylene glycol, wherein:

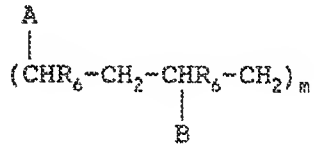
R_1 - $R_{10}(CH_2CH_2O)_nCH_2CH_2X$-, or -OH, and R_{10} = T-X- where X is at the 2'-position on the taxol side chain;

R_2 = -OH or $R_{11}(CH_2HC_2O)_nCH_2CH_2X$-, and R_{11} = T-X- where X is at the 7-position on the taxol ring structure;

where R_1 and R_2 do not simultaneously comprise a polyethylene glycol;

and n is a number between, and including, 5 and 2500.

6. The composition of claim 3 in which the polyethylene glycol is a branched polyethylene glycol, and the taxol-polyethylene glycol derivative has the general formula:



wherein A = $R_{12}(CH_2CH_2O)_nCH_2CH_2$-Y- and R_{12} = HO-, or alkoxy, or aryloxy, or T-X-;

wherein B = T-X-$(CH_2CH_2O)_pCH_2CH_2$-Y-;

wherein R_6 = -H or $-CH_3$;

wherein X is located at either the 2' position of the taxol side chain or the 7 position of the taxol ring structure but not both simultaneously;

wherein Y is a covalent linking group independently selected from the same groups as X;

and m, n, and p are numbers between, and including, 5 and 2500.

7. The composition of claim 3 in which the polyethylene glycol is a star polyethylene glycol, and the taxol-polyethylene glycol derivative has the general formula;

$$[T\text{-}X\text{-}(CH_2CH_2O)_nCH_2CH_2]_q\text{-(central core)}$$

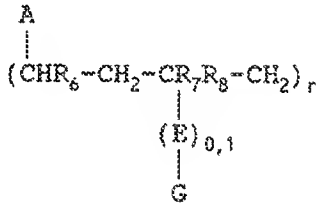
wherein X is located at either the 2' position of the taxol side chain or the 7 position of the taxol ring structure but not both simultaneously;

wherein q is the number of arms of a linear polyethylene glycol attached to a central core;

wherein n is a number between, and including, 5 and 2500;

and q is a number between, and including, 2 and 100.

8. The composition of claim 3 in which said water-soluble polymer is a copolymer of a linear polyethylene glycol and a functional monomer, the resultant copolymer-taxol derivative having the general formula;



wherein $A = R_{12}(CH_2CH_2O)_nCH_2CH_2\text{-}Y\text{-}$ and R_{12} = HO-, or alkoxy, or aryloxy, or T-X-;

wherein R_6 = -H or $-CH_3$;

wherein Y is a covalent linking group independently selected from the same groups as X;

wherein R_7 and R_8 are selected from H, or alkyl, or aryl;

wherein E is optionally selected from alkyl or aryl;

wherein G = -W-T, wherein W is a covalent linking group independently selected from the same groups

as X, and T is linked to E through W.
wherein W is located at either the 2' position of the taxol side chain or the 7 position of the taxol ring structure but not both simultaneously; and r is a number between, and including, 5 and 2500.

9. The composition of claim 6 in which a fraction of taxol substituents are replaced with unsaturated groups capable of undergoing free radical polymerization.

10. The composition of claim 7 in which a fraction of taxol substituents are replaced with unsaturated groups capable of undergoing free radical polymerization.

11. The composition of claim 8 in which a fraction of taxol substituents are replaced with unsaturated groups capable of undergoing free radical polymerization.

12. The composition of claim 6 in the form of a crosslinked insoluble gel containing covalently bound taxol.

13. The composition of claim 7 in the form of a crosslinked insoluble gel containing covalently bound taxol.

14. The composition of claim 8 in the form of a crosslinked insoluble gel containing covalently bound taxol.

15. A water-soluble drug-polymer conjugate having the general formula P-X-D;

wherein the polymer P is water-soluble;

wherein the drug D, is by itself water-insoluble or poorly water-soluble;

and the drug and polymer are linked by the covalent linkage X.

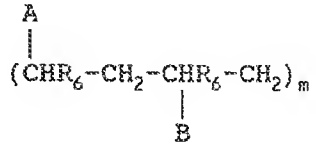
16. The composition of claim 15 in which said water-insoluble or poorly water-soluble drug D is taxol or amphotericin B.

17. The composition of claim 15 in which said water-soluble polymer is crosslinked to form an insoluble gel.

18. A method of solubilizing a water-insoluble or poorly water-soluble drug by attaching to a water-soluble polymeric carrier by a covalent linkage.

19. The method of 18 in which said water-insoluble or poorly water-soluble drug is taxol or amphotericin B.

20. A water-soluble drug delivery system comprising water-soluble polymers of polyethylene glycol conjugated to a drug D by a covalent linking group, the drug-polymer conjugates having the general formulas:



wherein the water-soluble polymer is a branched polyethylene glycol;

wherein said drug is linked to said water-soluble

polymer by said covalent linking group denoted by X;

wherein A = $R_5(CH_2CH_2O)_nCH_2CH_2$-Y- and R_5 = HO-, or alkoxy, or aryloxy, or D-X-;

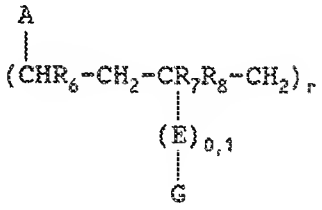
wherein B = D-X-$(CH_2CH_2O)_pCH_2CH_2$-Y-;

wherein R_6 = -H or $-CH_3$;

wherein Y is independently selected from the same groups as X;

wherein m, n, and p are numbers between, and including, 5 and 2500;

OR



wherein the water-soluble polymer is a branched copolymer of a linear polyethylene glycol and a functional monomer;

wherein said drug is linked to said water-soluble polymer by covalent linking groups X or W;

wherein A and R_6 are defined as above;

wherein R_7 and R_8 are selected from -H, $-CH_3$, alkyl, or aryl;

wherein E is optionally selected from alkyl or aryl;

and G = -W-D, wherein W is a covalent linking group independently selected from the same groups as X, and D is linked to E through W;

wherein r is a number between, and including 5 and 2500;

OR

[D-X-$(CH_2CH_2O)_nCH_2CH_2]_q$-(central core)

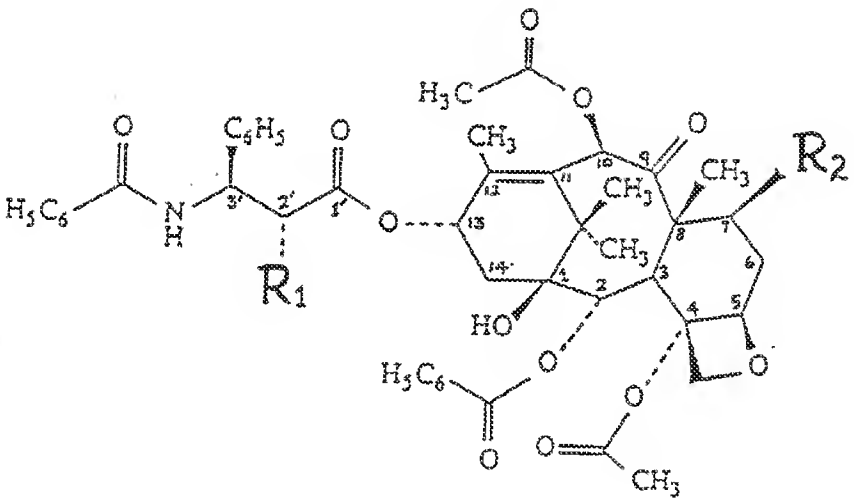
wherein the water soluble polymer is a star polyethylene glycol;
wherein said drug is linked to said water-soluble polymer by said covalent linking group denoted by X;
wherein q is the number of arms of a linear polyethylene glycol attached to a central core and q is a number between, and including, 2 and 100;
and n is defined as above.

21. The composition of claim 20 wherein said drug D is water-insoluble or poorly water-soluble.

22. The composition of claim 21 as a means of solubilizing said water-insoluble or poorly water-soluble drug in water.

23. The composition of claim 21 in which said water-insoluble or poorly water-soluble drug is taxol or amphotericin B.

FIGURE 1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H3C
O
CH3
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R2
C6H5
O
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H
3'
2'
1'
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10
9
CH3
CH3
8
7
6
CH3
14
1
2
3
4
5
R1
HO
O
O
H5C6
O
O
O
O
CH3

INTERNATIONAL SEARCH REPORT

Int. ..tional application No.
PCT/US93/05344

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07D 305/14,
US CL :549/510, 549/511

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : NONE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, taxol, Polyethylene glycol

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,534,899 (Sears), 13 Aug. 1985	1-23
Y	US, A, 5,059,699 (Kingston et al.), 22 Oct., 1991.	1-23
Y	"Polymer Preprints", 31, 1990, Nathan et al., Polyethylene Glycol-Lysine Copolymer: New Biocompatible Polymers For Biomedical Application", pp. 213-214.	1-23
Y	"Eur. Polym. J.", Vol. 19, No. 12, 1983, Zalipsky et al., Attachment of Drugs to Polyethylene Glycols, pp. 1177-1183	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 1993	Date of mailing of the international search report 09 SEP 1993
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INTERNATIONAL SEARCH REPORT

national application No.
PCT/US93/05344

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	"J. Med. Chem.", 24, 1981, Cecchi et al., Synthesis and Pharmacological Evaluation of Poly (oxyethylene) Derivatives of 4-Isobutyl phenyl-2-propionic Acid (Ibuprofen), pp. 622-625	1-23
Y	"J. Med. Chem.", Vol. 16, No. 5, 1973. Zilkha et al., Polyethylene Glycol Derivatives of Procaine, pp. 573-574.	